

WHAT IS CLAIMED IS:

1. A marker molecule of the formula I:

Segment A—L—Segment B

wherein,

Segment A is a labeled molecule;

L is a linker or a bond; and

Segment B is a protein or nucleic acid.

2. The marker molecule of claim 1, wherein said Segment A comprises at least two or more labeled amino acids.

3. The marker molecule of claim 1, wherein said label is selected from the group consisting of chromophores, fluorophores, and UV absorbing groups.

4. The marker molecule of claim 1, wherein L is a peptide bond.

5. The marker molecule of claim 2, wherein said labeled amino acid is a lysine.

6. The marker molecule of claim 1, wherein said Segment A comprises about one to about one hundred covalently linked amino acids.

7. The marker molecule of claim 1, wherein said Segment A comprises about five to about fifty covalently linked amino acids.

8. The marker molecule of claim 1, wherein said Segment A comprises about ten to about thirty covalently linked amino acids.

9. The marker molecule of claim 1, wherein said Segment A comprises 15 covalently linked amino acids.

10. The marker molecule of claim 1, wherein said Segment B has a molecular weight from about 3,000 daltons to about 250,000 daltons and a pI from about 2 to about 12.

11. A marker molecule composition comprising two or more marker molecules of claim 1.

12. The marker molecule composition of claim 11, wherein the two or more marker molecules have different molecular weights and/or isoelectric points (pI).

13. A method of separating one or more molecules present in a sample in a matrix, the method comprising adding the marker molecule composition of claim 11 to the sample containing one or more molecules, applying the sample to the matrix, and subjecting matrix to electric field.

14. A method of separating one or more molecules present in a sample, the method comprising adding the marker molecule composition of claim 11 to the sample containing one or more molecules, applying the sample to a matrix, and separating the one or more molecules.

15. The method of claim 13, further comprising, after subjecting the matrix to an electric field, detecting the molecular markers and comparing the position of the labeled molecular markers to the position of said one or more molecules.

16. ✓ A method of preparing a marker molecule, the method comprising:

- (a) labeling a molecule; and
- (b) ligating the molecule to a protein and/or nucleic acid of known molecular weight, wherein the molecule or protein and/or nucleic acid contains an  $\alpha$ -thioester and the other contains a thiol-containing moiety.

17. The method of claim 16, further comprising:

- (c) repeating (a)-(b) one or more times to obtain a number of labeled marker molecules of different molecular weights and pIs; and
- (d) combining the labeled marker molecules having different molecular weights and pIs.

18. The method of claim 16, wherein said thiol-containing moiety is a 1-phenyl-2-mercaptoethyl group.           

19. ✓ A method of preparing a marker molecule, comprising:

- (a) labeling a molecule comprising an amino-terminal cysteine residue; and
- (b) ligating the molecule with a protein and/or nucleic acid of known molecular weight and comprising a  $C_{\alpha}$ -thioester.

20. The method of claim 19, further comprising:

- (c) repeating (a)-(b) one or more times to obtain a number of labeled marker molecules of different molecular weights and pIs; and
- (d) combining the labeled marker molecules having different molecular weights and pIs.

21. A method of labeling a marker molecule, comprising:

- (a) attaching a first amino acid to a solid phase;

(b) coupling said first amino acid to a second amino acid protected by blocking groups resulting in a chain of amino acids, wherein said blocking groups are removed before the addition of amino acids;

(c) extending the length of the chain by solid phase synthesis with additional amino acids, wherein said chain comprises at least one labeled amino acid, resulting in a labeled oligopeptide;

(d) releasing the labeled oligopeptide from the solid phase;  
and

(e) ligating the labeled oligopeptide with a protein of known molecular weight.

22. The method of claim 21 wherein said labeled oligopeptide comprises one, two or more amino acids modified with a label.

23. The method of claim 21 wherein said blocking groups are selected from the group consisting tert-butyloxycarbonyl (BOC), 9-fluorenylmethoxycarbonyl (FMOC) and derivatives thereof.

24. A method of characterizing one or more proteins comprising:

(a) electrophoresing one or more proteins in a matrix with at least one marker molecule of claim 1; and

(b) comparing the migration of the one or more proteins with the migration of the at least one marker molecule; and

(c) optionally, determining the isoelectric point (pI) and/or molecular weight of the one or more proteins.

25. A method of characterizing one or more molecules comprising:

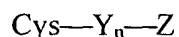
(a) separating one or more molecules in a matrix with at least one marker molecule of claim 1; and

(b) comparing the migration of the one or more molecules with the migration of the at least one marker molecule; and

(c) optionally, determining the isoelectric point (pI) and/or molecular weight of the one or more molecules.

26. The method of claim 24 wherein said gel is a two-dimensional electrophoresis gel.

27. ✓ A peptide having the formula II:



wherein,

Y is one or more amino acid selected from the group consisting of alanine, arginine, aspartic acid, asparagine, cysteine, glutamic acid, glutamine, glycine, histidine, iso-leucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine and/or a non-natural amino acid;

Z is a C-terminal amino acid and/or non-natural amino acid;  
and

n=1-100.

28. The peptide of claim 27, wherein Y is labeled with one or more chromophores, fluorophores, or UV absorbing groups.

29. The peptide of claim 27, having the following sequence:  
Cys-Asp-Asp-Lys(TMR)-Asp-Asp-Asp-Asp-Leu-Ala-Asp-Asp-Asp-  
Lys(TMR)-Asp-amide (SEQ ID NO:6).

30. The peptide of claim 27, having the following sequence:  
Cys-Asp-Lys(TMR)-Asp-Ala-Asp-Asp-Leu-Ala-Asp-Leu-Asp-Lys(TMR)-  
Asp-Ala-amide (SEQ ID NO:7).

31. The peptide of claim 27, having the following sequence:

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Cys-Gly-Lys(TMR)-Ser-Gly-Ser-Gly-Lys-Ser-Gly-Lys-Gly-Lys(TMR)-Ser-Gly-amide (SEQ ID NO:8).

32. The peptide of claim 27, having the following sequence:  
Cys-Ala-Lys(TMR)-Leu-Lys-Ala-Lys-Ala-Lys-Leu-Ala-Lys-Lys(TMR)-Leu-Ala-amide (SEQ ID NO:9).

33. The peptide of claim 27, having the following sequence:  
Cys-Lys-Lys(TMR)-Lys-Ala-Lys-Leu-Lys-Ala-Lys-Lys-Lys-Lys(TMR)-Ala-amide (SEQ ID NO:10).

34. The peptide of claim 27, further comprising a tag molecule.

35. The peptide of claim 34, wherein said tag molecule is selected from the group consisting of biotin, fluorescein, digoxigenin, polyhistidine and derivatives thereof.

36. A protein marker kit comprising a carrier having in close confinement therein at least one container where a first container contains at least one marker molecule of claim 1.

37. The protein marker kit of claim 36, further comprising instructions for use of kit components.

38. The protein marker kit of claim 36, further comprising a pre-cast electrophoresis gel.